Research Article

Cryptic niche switching in a chemosymbiotic gastropod

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Running head

Cryptometamorphosis

Abstract

Life stages of some animals, including amphibians and insects, are so different that they have historically been seen as different species. ‘Metamorphosis’ broadly encompasses major changes in organism bodies and, importantly, concomitant shifts in trophic strategies. Many marine animals have a biphasic lifestyle, with small pelagic larvae undergoing one or more metamorphic transformations before settling into a permanent, adult morphology on the benthos. Post-settlement, the hydrothermal vent gastropod *Gigantopelta chessoia* experiences a further, cryptic metamorphosis at body sizes around 5-7 mm. The terminal adult stage is entirely dependent on chemoautotrophic symbionts; smaller individuals do not house symbionts, and presumably depend on grazing. Using high resolution x-ray microtomography to reconstruct the internal organs in a growth series, we show this sudden transition in small but sexually mature individuals dramatically reconfigures the organs, but is in no way apparent from external morphology. We introduce the term ‘cryptometamorphosis’ to identify this novel phenomenon of a major body change and trophic shift, not related to sexual maturity, transforming only the internal anatomy. Understanding energy flow in ecosystems depends on the feeding ecology of species; the present study highlights the possibility for adult animals to make profound shifts in biology that influence energy dynamics.

Background

Metamorphosis in animals involves profound physical transformation in the morphology of an individual, linked to significant changes in ecology and behaviour. For example, holometamorphic insects and amphibians have radically different body plans, niche, and ecology in their larval and adult stages, often occupying distinct trophic levels. Marine invertebrates commonly have a biphasic life history, with dispersing larval stages in the plankton and a post-settlement stage following a terminal metamorphosis. Some ontogenies are even more dramatic in that they have multiple metamorphoses, a series of larval stages each with very different morphology. Many larval stages were historically described as different genera from the adult animals, such as nauplius and zoea larval stages of crustaceans [1, 2]. 'Hypermetamorphosis' has been used to describe animals with multiple and drastically different larval phases, a term generally used for insects and fish [3]. Although metamorphosis appears to occur synchronously in external morphology and internal anatomy, the shift in external morphology may be de-coupled from changes in the internal organs [4]. Anatomical transitions often require that animals go through a non-feeding transitional stage, such as the insect pupa or the metamorphosing larva in fish [5, 6].

A metamorphic transition usually alters the trophic interactions of an organism. In marine invertebrates, larvae and adults of benthic species are spatially as well as morphologically separated. In freshwater systems, larvae and adults can co-occur, so communities are often assessed by feeding guild rather than taxonomy [7]. Modelling energy flow in all food webs necessarily depends on simplifying assumptions, such as consistency in metabolic scaling with biomass [8, 9]; however, general patterns could be occluded by additional, unrecognised major trophic shifts within adult or larval life stages. Reconstructing food webs in key marine ecosystems is difficult because they generally have longer food chains than terrestrial ecosystems [10]. Deep-sea hydrothermal vent ecosystems are highly productive and host a large biomass of comparable density to tropical reefs, but have a relatively simple, reduced trophic complexity [11]. Hydrothermal vents thus offer an important model system to test ideas of trophic ecology and energy flow, if we fully understand the trophic positions and niche switching dynamics of their constituent species.

A large bodied gastropod endemic to hydrothermal vents in the Southern Ocean was reported to undergo a substantial transition [12], from grazing to total dependency on

internal endosymbiotic bacteria, at an unusually late stage of post-settlement life. *Gigantopelta chessoia* Chen *et al*., 2015 is a large (body length ~50 mm) peltospirid gastropod first discovered in 2012 [13, 14]. This gastropod is unusual in having a large, specialised bacteria-housing organ or 'trophosome' inside the body, ontogenetically derived from an oesophageal gland [12]. Dependency on microbes allows the animals to reach comparably large body sizes [15]. Only two gastropod genera are known to have this specialised organ, all other vent molluscs with internal chemosymbionts house the microbes in their gill tissue [12].

In small individuals of *Gigantopelta chessoia*, where the gonad is apparently completely developed, the oesophageal gland is very small and not enlarged into a 'trophosome' [12]. We hypothesised that this unusual metamorphic shift, the later enlargement of the 'trophosome', is associated with a change in diet from grazing to total dependency on internal symbionts, with the inoculation of chemosymbionts occurring during this transition. Surprisingly, the small individuals (without a developed 'trophosome') and larger individuals, are otherwise identical in external morphology. In order to examine the arrangement of the internal organs non-invasively, we used synchrotron x-ray computed tomographic (CT) imaging of the soft parts across post-settlement ontogeny. In the present study, our aims were to determine whether this transition was a gradual isometric growth of particular organ, or a more transformative change at a specific stage of growth that could be considered a cryptic, internal metamorphosis.

Methods

Specimens of *G. chessoia* were collected from chimney surfaces using a suction sampler on the remotely operated vehicle (ROV) *Isis* during the RRS *James Cook* expedition JC80 from the hydrothermal vent field at 2,644 m deep on segment E2 of the East Scotia Ridge [12, 13]. Six post-settlement specimens representing post-settlement ontogeny (body lengths: 2.2 mm, 3.5 mm, 5.1 mm, 7.8 mm, 12.2 mm, 23.0 mm) were selected from specimens fixed and stored in 10% buffered formalin, and then post-stained with 1% iodine solution for five days prior scanning by synchrotron hard x-ray micro computed tomography (micro-CT).

Specimens were scanned at one of two different synchrotron facilities, to take advantage of differences in the optimised fields of view of optics. Scans of small specimens (below

7 mm body length) were completed at beamline 8.3.2 at the Advanced Light Source (ALS), Lawrence Berkeley National Laboratory (Berkeley, USA). Larger specimens (above 7 mm) were visualised in hutch #3 of the beamline BL20B2 at the SPring-8 (Hyogo, Japan). At the ALS, scans were done using monochromatic X-rays at 23 keV, with a home-built lens system with Mitutoyo 5x or 2x lenses and a LuAG:Ce 150 or 500 micron thick scintillator. A PCO.edge sCMOS camera was used, yielding effective pixel sizes of 1.72 µm for the 5x lens or 3.44 µm for the 2x lens. Samples were rotated through 180 degrees while 2049 images were collected. Reconstruction was performed with Tomopy and Xi-cam (alpha release, 2017, http://www.camera.lbl.gov/xi-cam-interface). At the SPring-8, similar scans were performed at 25 keV [16] using a Hamamatsu Photonics K.K. CCD camera [17], yielding 1860 images with effective pixel sizes of 6.5 µm or 13.16 µm.

The resulting images were processed in Adobe Photoshop CC for contrast enhancement and then imported into Amira v5.3.3 (FEI Visualisation Sciences Group), aligned into a single stack and highlighting the materials of interest. Post-processing including surface rendering and smoothing to generate the final tomographic model following previously published methods [18, 19].

For transmission electron microscopy (TEM), a portion of the oesophageal gland and gill leaflet from small (2.6 mm body length) and large (25.8 mm body length) *Gigantopelta* specimens fixed in 10% buffered formalin were first sliced to approximately 0.2-0.4 mm thick with a razor blade after washing with filtered sea water. In order to best preserve the ultrastructure, we employed an ice-free high-pressure freezing/freeze substitution (HPF/FS) technique [20] (Leica EM-PACT2). Frozen samples were post-fixed with 2% OsO_4 in acetone for 3-4 days at -80°C, and then gradually brought to room temperature, rinsed with acetone, and embedded in epoxy resin (TAAB, Aldermaston, U.K.). An ultramicrotome (Leica EM-UC7) was used to make ultrathin sections (70 nm) which were then post-stained with 2% uranyl acetate and lead solution (0.3% lead nitrate and 0.3% lead acetate, Sigma-Aldrich). Transmission electron microscopy (TEM) was performed using a Tecnai 20 TEM (FEI) at an acceleration voltage of 120 kV.

Results & Discussion

We reconstructed the whole digestive systems of six *Gigantopelta* specimens across a

size range spanning an order of magnitude, from 2.2 mm to 23.0 mm (Figure 1). It is clear from the reconstructions that during ontogeny the oesophageal gland suddenly expands and takes over the visceral mass between body sizes of 5.1 mm to 7.8 mm, a small difference compared to the normal adult body size of ~ 50 mm. From quantification of organ volumes (Figure 2), the overall relative volume of the digestive system within the total body does not change significantly during growth, and increases isometrically as approximately 20% of the body volume. Meanwhile, the proportion of the digestive system occupied by oesophageal gland dramatically increases at the transition stage and then grows isometrically with body size (Figure 2).

In early post-settlement stages, the oesophageal gland largely contains open space and lacks endosymbionts (Figure 2a), whereas after the transition the same tissue expands to form the 'trophosome' and it is filled with bacteriocytes (Figure 2b). Although previous workers speculated that the gill epidermis may also house endosymbionts [21], we also examined the gill epidermis via TEM and found that the gill lacks any endosymbionts both before and after the transition stage. Taken together, this is evidence that a transformation in anatomy is accompanied by a transition in feeding ecology. We interpret this post-settlement transition in *Gigantopelta* as a metamorphosis, which drastically alters its internal anatomy in changing the entire digestive system to enable a niche switching to a terminal adult stage with total dependency on intracellular chemosymbionts.

We consider the term 'cryptometamorphosis' to be a suitable descriptor for this phenomenon. There is some disagreement about the definition of what is considered 'metamorphosis', but broad consensus is that it involves both a change in morphology and life history, which shifts the 'adaptive landscape' in that the selective pressures acting at the individual level or group level are altered [22]. In this sense, although the morphological changes in are internal rather than external, the post-settlement transition in *Gigantopelta* qualifies as a true metamorphosis. There are cryptic aspects to any metamorphosis, with anatomical transitions happening prior to external changes, such as in butterflies prior to their emergence from a chrysalis [23]; however, in all other known cases, metamorphoses are associated with profound and correlated changes in both external and internal morphology, even if internal and external reconfigurations may be decoupled [4]. In *Gigantopelta*, there is no external evidence in either the shell or the body showing the change to the terminal form has been completed, yet the internal reconfiguration changes the animal's autecology.

Differential allometric rates of growth among organs can be induced by life history in permanent or transient change; however, differences in proportions alone are not metamorphosis. A metamorphosis, such as we see in *Gigantopelta*, includes the transformation in organ structure and function as well as size. It is not uncommon for animals to experience changes in trophic niche or target prey size with increasing size or allometric change over ontogeny. In the yellowfin tuna *Thunnus albacares* (Bonnaterre, 1788) subadults change diets significantly at sizes around 45-50 cm forklength, from feeding on plankton to feeding primarily on other teleosts [24]. This type of trophic shift, however, has no metamorphic quality in terms of either internal or external morphological transition. Metamorphosis is also conceptually and functionally different to changes associated with phenology or sexual maturity, which are not necessarily linked with shifts in niche occupation. Small-sized *Gigantopelta*, before the transformation, already have a well-developed gonad and should therefore be regarded as sexually mature [12], their cryptometamorphosis is thus not apparently related to sexual maturity.

Nutritional dependency on endosymbionts is often correlated with dramatic increases in size and growth rate of marine lineages, a trend which could apply to *Gigantopelta* [15]. Metabolic rates and growth in ectotherms are also strongly dependent on temperature, and vent endemic animals mostly live in temperatures much higher than the surrounding seawater [11], with thermal regimes comparable to the subtropics. Some chemosymbiotic holobionts such as the giant vent tubeworm *Riftia* [25, 26] and the giant vesicomyid clam *Calyptogena* [27] are characterised by extremely high growth rates, faster than any other marine invertebrates [28]. There is also an evident influence of hosting symbionts on gigantism in other environments, such as photosymbiotic living (*Tridacna*) and fossil (rudist) giant bivalves that represent some of the largest benthic marine invertebrates that ever lived [29]. Although it is not universally true that all holobionts are necessarily giants [30, 31], this trend toward increased body size attributable to endosymbionts is seen in a variety of reducing environments. Recently the giant shipworm *Kuphus polythalamius* (Linnaeus, 1767) which inhabits shallow water reducing muds was found to exhibit thioautotrophic endosymbionts [32], and endosymbionts were likely also responsible for gigantism in its fossil relatives that were originally mistakenly described as a dinosaur [33].

Chemosymbiotic holobiont vent snails (*Gigantopelta*, *Chrysomallon*, *Alviniconcha*, and *Ifremeria*) likewise grow much larger than their close relatives in their respective families

[12, 34]. The pre-cryptometamorphosis size of *Gigantopelta chessoia* is approximately the average adult size of other members of its subclass Neomphalina, and most others in the group feed by grazing or deposit-feeding [35]; change from a grazer to a holobiont at a late stage likely allows *Gigantopelta* to reach a much larger size at the terminal adult stage and might therefore increase reproductive output. This is evidence that symbiosis, a key feature of vent ecosystems, is advantageous to the host lineages no matter at what stage the symbiont acquisition occurs.

Deep-sea bathymodioline mussels, a family which includes species in multiple deep sea reducing environments such as woodfalls and hydrothermal vents, may be another potential example of cryptometamorphosis [36]. These mussels probably initially settle as solely filter-feeding young, and speculatively transition later to reliance on endosymbionts [37, 38]. Fully-grown adult mussels house endosymbiotic bacteria in their gills, and it remains unclear at what point inoculation occurs, or whether it is accompanied by any other changes to gills or the digestive organs. Giant vent tubeworms in the genus *Riftia* initially settle as later-stage trochophores with a complete larval digestive system feeding on microbes, and then are inoculated with endosymbionts through the skin during metamorphosis to the adult benthic form; these bacteria migrate across several layers of host tissue to infect mesodermal tissue which develops into the trophosome entirely independent of the digestive tract [39]. In later juveniles, the trophosome has already completed development, and therefore the *Riftia* endosymbiont inoculation process can be considered part of its metamorphosis process from larva to juvenile. Unlike *Riftia*, the 'trophosome' of *Gigantopelta* is clearly derived from oesophageal gland tissue, part of the digestive system, and its development into a bacteria-housing organ happens as a separate metamorphosis.

The trophosome-like structure that characterises *Gigantopelta* is only found in one other mollusc genus, also in the family Peltospridae: the Scaly-foot Gastropod *Chrysomallon squamiferum* Chen *et al*., 2015, from the Indian Ocean [40]. *Gigantopelta* and *Chrysomallon* have superficially similar but evolutionarily convergent adaptations to exploit chemosynthetic resources in hydrothermal vent ecosystems [12]. Juveniles of *Chrysomallon* have a hypertrophied 'trophosome' already at 2 mm body length, at a point after settlement but before any development of the gonads [35]. *Chrysomallon* also has a single oesophageal gland, but it is symmetrical and may be derived from a single gland or a fused pair [35]. Other smaller, non-symbiotic peltospirids such as *Peltospira* and *Rhynchopelta* have paired oesophageal pouches that are not used to house bacteria [41].

Gigantopelta only possesses a single oesophageal gland that is asymmetrical in all growth stages (Figure 1). The isometric growth and lack of cryptometamorphosis in *Chrysomallon* reinforces the evidence that the 'trophosomes' in *Gigantopelta* and *Chrysomallon* represent parallel adaptive evolution and not a feature of the family Peltospiridae.

Both *Gigantopelta* and *Chrysomallon* have an elaborate circulatory system, including a pumping muscular heart to transport hydrogen sulfide and oxygen to their endosymbionts [12, 35]. The development of the 'trophosome' is fundamental to housing endosymbionts, so other related anatomical adaptations in the circulatory system of *Gigantopelta* are directly related to supporting its niche switching. It is unclear how different holobiont lifestyles impact oxygen metabolism or metabolic scaling, as there is apparently little difference between the metabolic rate of holobiont gastropods housing bacteria in a 'trophosome', or within the gills [42]. What is important to consider in the case of *Gigantopelta*, and possibly other species in especially chemosymbiotic ecosystems, is that there is a substantial difference in energy flow between direct consumption of nutrients through chemosymbiosis and food resources that originate outside the body. Evolution of this lineage exploits the energy efficiency of symbiosis, but the potential advantages are conferred at a surprisingly late stage in development that is unique among vent holobionts.

Vent animals have many curious adaptations, but the limited species diversity and relatively short interacting chains make vents a good model system for understanding trophic dynamics [11], if we have multiple lines of evidence to understand the true energy sources of the animals at different life stages in each ecosystem. The findings in this study advance the understanding the ecology and evolution of chemosymbiotic organisms by demonstrating that different holobiont lineages with similar symbiont-housing strategies may have drastically different symbiont inoculation processes. Importantly, the newly revealed phenomenon of cryptometamorphosis expands our concept and horizon of animal metamorphosis and niche switching.

Data, code and materials

The datasets supporting the results of this article are included within the article and its additional file, also available on Dryad [43].

Ethics

Study species were gastropod molluscs collected within the South Georgia and the South Sandwich Island (SGSSI) Maritime Zone, with a scientific research permit from the Government of SGSSI for the RRS *James Cook* expedition JC80. Additional permits were granted by the Foreign and Commonwealth Office, U.K. under the Antarctic Act 1994 to undertake an expedition (Section 3, No. S3-03/2012) and for RRS *James Cook* to enter Antarctica (Section 5, No. S5-05/2012). Animals collected were immediately preserved in formalin after recovery and no live experiments were carried out.

Authors' contributions

CC conceived the study, participated in the design of the study, collected the specimens used, carried out the lab work, participated in data analysis, and drafted the manuscript with JDS; KL collected specimens and field data, and assisted with data interpretation; KU carried out the TEM histology work and assisted with data interpretation; JDS conceived the study, designed the study, contributed to lab work, participated in data analyses, and drafted the manuscript with CC. All authors gave final approval for submission and publication.

Competing interests

We have no competing interests.

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Figure Legends

Figure 1. 3D reconstructions of the digestive system in *Gigantopelta chessoia* across post-settlement ontogeny, at body lengths (a) 2.2 mm, (b) 3.5 mm, (c) 5.1 mm, (d) 7.8 mm, (e) 12.2 mm, (f) 23.0 mm. The 'trophosome' is indicated by yellow, shades of blue indicate other parts of the digestive system, including buccal mass, oesophagus, stomach, digestive gland, and intestine. Scale bars = 1 mm for all parts.

Figure 2. Transmission electron micrographs of the 'trophosome' or oesophageal gland in post-settlement *Gigantopelta chessoia* at body length (a) 2.6 mm and (b) 25.8 mm; as well as (c) the relationship between body length and the relative percent body volume of the whole digestive system combined (dashed grey line and triangles), the 'trophosome' (black line and filled circles), and the digestive system not including the 'trophosome' (light grey line and squares).

Additional Files

Video S1. A video illustrating a hypothesised scenario of isometric growth post-settlement in *Gigantopelta chessoia* versus the observed cryptometamorphosis, showing changes in the anatomy of the digestive system around the point of transition (3.5 mm to 12.2 mm body length). Simulated growth and rendering was done in Blender v2.79b.

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